



**Reduced radial displacement of the Gastrocnemius Medialis muscle following electrically elicited fatigue**

Journal:	<i>Journal of Sport Rehabilitation</i>
Manuscript ID:	JSR.2014-0325.R1
Manuscript Type:	Original Research Report
Keywords:	muscle contractile properties, maximal voluntary contraction, TMG, passive muscle tension, peripheral fatigue
Copyright Human Kinetics. This version is as accepted for publication in Journal of Sport Rehabilitation	

SCHOLARONE™  
Manuscripts

Peer Review

## 1 Abstract

2 **Context:** Assessments of skeletal muscle functional capacity often necessitate maximal  
3 contractile effort, which exacerbates muscle fatigue or injury. Tensiomyography (TMG) has  
4 been investigated as a means to assess muscle contractile function following fatigue;  
5 however observations have not been contextualised by concurrent physiological measures.

6 **Objective:** The aim of the present investigation was to measure peripheral fatigue-induced  
7 alterations in mechanical and contractile properties of the plantar flexor muscles through  
8 non-invasive TMG concurrently with maximal voluntary contraction (MVC) and passive  
9 muscle tension (PMT) in order to validate TMG as a gauge of peripheral fatigue. **Design:**  
10 Pre- and post-test intervention with control. **Setting:** University laboratory. **Participants:**  
11 Twenty-one healthy male volunteers. **Interventions:** Subjects plantar flexors were tested for  
12 TMG parameters, along with MVC and PMT, before and after either a 5 minute rest period  
13 (control) or a 5 minute electrical stimulation intervention (fatigue). **Main Outcome**  
14 **Measures:** Temporal (contraction velocity) and spatial (radial displacement) contractile  
15 parameters of the Gastrocnemius Medialis were recorded through TMG. MVC was  
16 measured as an indicator of muscle fatigue and PMT was measured to assess muscle  
17 stiffness. **Results:** Radial displacement demonstrated a fatigue-associated reduction ( $3.3 \pm$   
18  $1.2$  vs.  $4.0 \pm 1.4$  mm vs,  $p=0.031$ ), while contraction velocity remained unaltered.  
19 Additionally, MVC significantly declined by  $122.6 \pm 104$  N ( $p<0.001$ ) following stimulation  
20 (fatigue). PMT was significantly increased following fatigue ( $139.8 \pm 54.3$  vs.  $111.3 \pm 44.6$   
21 N,  $p=0.007$ ). **Conclusion:** TMG successfully detected fatigue, evident from reduced MVC,  
22 by displaying impaired muscle displacement, accompanied by elevated PMT. TMG could

23 be useful in establishing fatigue status of skeletal muscle without exacerbating the  
24 functional decrement of the muscle.

25

26 **Key words:** muscle contractile properties, maximal voluntary contraction, TMG, passive  
27 muscle tension, peripheral fatigue

28

## 29 Introduction

30 Muscle fatigue is characterised by a decrease in the external force or torque generating  
31 capacity,<sup>1</sup> and/or by impairment in peak power output.<sup>2</sup> The manifestation and magnitude  
32 of this reduced function depends upon multiple factors including the muscle contraction  
33 mode,<sup>1</sup> the nature of the fatigue protocol<sup>3</sup> and the source of the fatigue.<sup>4</sup> Fatigue-related  
34 alterations of skeletal muscle can be observed, amongst other factors, by changes in its  
35 contractile and mechanical properties.

36 Since fatigue is a condition that affects both athletic performance and clinical mobility, the  
37 need for a valid monitor of muscle response is important to enable optimal management of  
38 athletes and patients. In situations of muscle fatigue, or indeed musculoskeletal injury, it is  
39 impractical to assess muscle function through a measure which makes use of voluntary  
40 efforts (i.e. MVC), due to centrally mediated inhibition.<sup>5</sup> Furthermore, the potential for  
41 aggravation of any damage to the musculoskeletal unit cannot be ruled out. Having been  
42 developed over the last 15 years, tensiomyography (TMG) is a portable and non-invasive  
43 means of measuring muscle response through combined use of sub-maximal (below  
44 voluntary maximal activation) electrical stimulus and a digital displacement sensor,<sup>6-8</sup> similar

45 to that used in mechanomyography.<sup>9</sup> TMG records spatial and temporal parameters of the  
46 radial displacement of the muscle belly in response to electrical stimuli<sup>10</sup> and is reliable  
47 within<sup>11</sup> and between days.<sup>12</sup> Furthermore, TMG has also demonstrated good long-term  
48 stability following fatigue<sup>13</sup> and has displayed significant interclass correlation coefficient  
49 with decline and recovery of maximal voluntary contraction (MVC) following exercise-  
50 induced muscle damage.<sup>14</sup> **In particular muscle displacement (Dm) and contraction time**  
51 **(Tc) have shown greatest stability.**<sup>12</sup>

52 TMG has successfully detected fatigue-associated changes following ultra-endurance  
53 triathlon,<sup>15</sup> and resistance exercise.<sup>16</sup> However, these studies report inconsistent results in  
54 the fatigue-induced alteration of the TMG parameters, perhaps due to the vast differences  
55 in the fatigue protocols administered and the different muscles measured. Furthermore,  
56 previous studies have failed to relate the TMG alterations to any valid functional measure,  
57 such as maximal voluntary contraction (MVC) or passive muscle tension (PMT), which leaves  
58 the physiological interpretation of the TMG data open to question. Therefore, in order to  
59 effectively provide meaningful validation of TMG measurement to local fatigue it is  
60 important to overcome this limitation. In practical terms, sub-maximal TMG could offer an  
61 attractive measure for sport and medical practitioners in their assessment of muscle  
62 response and status following fatigue based activity without necessitating voluntary  
63 contractile effort.

64 Accordingly, the aim of the present investigation was to evaluate peripheral fatigue-induced  
65 alterations in mechanical and contractile properties of the Gastrocnemius muscle, as  
66 measured by TMG. MVC and PMT were measured before and after intervention, to quantify  
67 the extent of muscle fatigue, and allow us to better interpret changes in TMG response; to

our knowledge this has not been previously reported. It was hypothesised that a reduction in size and velocity of muscle displacement would indicate muscle fatigue in line with impairments in muscle function (decreased MVC) and elevated muscle stiffness (increased PMT). The findings of this study could help to establish TMG as a non-invasive alternative to quantify muscle fatigue.

## Methods

### *Participants*

Twenty-one healthy males with a mean ( $\pm$  SD) age, height, and mass of  $21.3 \pm 3.4$  years,  $182.0 \pm 6.1$ cm, and  $79.5 \pm 10.0$ kg, volunteered and gave their written informed consent to participate in this study. All participants were recreationally active and free from injury. Females were excluded from the study in order to maintain cohort homogeneity. The study was performed in accordance with the principles outlined in the *Declaration of Helsinki* and was approved by the local research ethics committee.

### *Design*

Mechanical and contractile properties of the right Gastrocnemius Medialis (GM) were monitored using TMG (BMC Ltd, Ljubljana). GM is one of the propulsive muscles, fundamental to different types of human locomotion and is located superficially, making it clearly measurable by TMG. Participants were also tested for PMT and MVC of the right plantar flexors. Testing was carried out on two occasions, one week apart, as illustrated in figure 1. Measurements were taken at a number of time points before and after either the control or fatigue intervention, according to the following order: TMG and PMT

(measurement 1, M1), warm-up, TMG and PMT + MVC (M2), either control or fatigue intervention in random order, TMG and PMT + MVC (M3). Both TMG and PMT measurements were recorded three minutes after the warm-up, and after the control or fatigue intervention, to limit the effects of post activation potentiation in the GM muscle.<sup>18</sup> Participants reported to the laboratory on the morning of each experimental trial in a fasted and rested state. Twenty-four hour dietary intake records were completed on the day preceding each trial, and participants were instructed to replicate their dietary intake before each visit.

*Warm-up*

Participants warmed up by cycling at a low intensity (75 Watts) on an electromagnetically braked cycle ergometer (Lode Ergometer, Netherlands) for 5 minutes at a cadence between 80 and 90 rpm.

*TMG protocol*

**TMG measurements were performed exactly as described by Ditroilo et al (2013).<sup>13</sup> Briefly, participants lay in a prone position on a padded bench. A foam pad, placed slightly proximal to the ankle joint, supported a knee flexion angle of around 5°. The digital displacement transducer (TMG–BMC Ltd, Ljubljana) was then positioned perpendicular to the muscle belly of the right GM with an initial pressure of  $1.5 \times 10^{-2}$  N/mm<sup>2</sup>, controlled by consistently retracting the spring-loaded transducer probe to 50% of its length. This measuring position was selected by first manually palpating the GM to locate the thickest part of the muscle and then later, if needed, the position was slightly adjusted to obtain the**

highest mechanical response with the least amount of co-activation when externally stimulated; co-activation was typically identified by a second peak in the TMG response curve. Once the appropriate position was obtained, it was marked with a permanent marker pen to ensure exact uniformity when the sensor was repositioned for subsequent measurements. The centre point of each of the 2 stimulating electrodes ( $5\text{cm}^2$ ) (Axelgaard, USA) was located approximately half way from the position of the sensor ( $\sim 5\text{cm}$ ) to the start of the respective GM proximal distal tendons. After each measurement these electrodes were left in place and unplugged to avoid any possible changes in muscle response via alterations in surface electrodes distance.<sup>10</sup> A single 1ms wide stimulation pulse was delivered, which applied initial current amplitude of 20mA. **This amplitude was progressively increased by 10mA increments until maximal response was obtained, i.e. no further displacement of the muscle belly could be produced as identified by a plateau in the twitch response curves.** In order to minimize the effects of fatigue and potentiation, rest periods of 10 seconds were allowed between each stimulation pulse. Typical maximal responses were observed at amplitude between 40 and 70mA and only the output data for that particular stimulation intensity were used for analysis. Figure 2 shows a typical TMG displacement/ time curve before and after the administration of the fatigue protocol. Output parameters were extracted and analysed from each maximal twitch response:<sup>10</sup> *Displacement* (Dm), the extent of maximal radial deformation (mm) of the muscle belly during contraction; *Contraction velocity* (Vc), the rate ( $\text{mm}\cdot\text{s}^{-1}$ ) of contraction between 10% and 90% of maximal displacement. **Raw data were extracted from the TMG software and Vc was calculated according to the formula:  $[Vc = Dm80/Tc]$  where Tc = contraction time between 10% and 90% of peak radial displacement of the muscle belly; Dm80 = the radial displacement occurring during the time period of Tc.**<sup>19</sup> Muscle contraction time (Tc) has

been widely reported in previous studies,<sup>10,15-16</sup> as the temporal change from 10%-90% of muscle Dm, providing a value relative to the spatial characteristics of each muscle. However, when assessing intramuscular alterations, i.e. pre- and post- fatigue, the significance of calculating Tc in this manner should be questioned. **Indeed, in the absence of signal latency, it is possible that a decrease in Dm could associate with a decrease in Tc, when calculated as described above. Apparent decreases in Tc, suggesting a faster twitch response, could be reported simply as a result of reduced overall muscle contraction (Dm).** It was therefore proposed that assessment of Vc could provide greater insight, when monitoring the fatigue status of a muscle.

#### **Maximal voluntary contraction (MVC) protocol**

**Plantar flexor isometric MVC was performed in an isokinetic dynamometer (Kin-Com, Chattanooga Group Inc., USA). The participant had their right foot fastened securely into the plantar flexion attachment and was also held in place using two securely fastened shoulder straps and a lap belt. A 90° ankle angle to the tibia was ensured for each subject (figure 3). Following two sub-maximal warm-up sets, participants each performed a 5-s MVC of the right plantar flexors. Three trials of the MVC were completed with 60s recovery between attempts. Participants were verbally motivated to ensure the greatest possible effort for the duration of all attempts.**

#### *Passive muscle tension (PMT) protocol*

**Measurements of PMT of the right plantar flexors were made on the same isokinetic dynamometer, with a set-up identical to the MVC protocol (figure 3). Participants were**



158 **instructed to completely relax once in position, and the mean passive force of the ankle**  
159 **flexed at 90° was recorded during a period of 15s, as a measure of passive muscle tension**  
160 **in the plantar flexors in a static position.**<sup>20</sup> A single measure was taken to determine PMT,  
161 as subsequent stretching of the ankle joint would cause an accumulative stretch effect. **An**  
162 **intra-session reliability, as measured by the intraclass correlation coefficient,  $\geq 0.80$  has**  
163 **been previously reported for this type of measurement.**<sup>21</sup>

#### 164 *Fatigue protocol*

165 **The fatigue intervention used in the current investigation differs from previous studies in**  
166 **this area**<sup>15,16</sup> **in a number of key ways. Firstly, fatigue was induced locally with a low**  
167 **frequency stimulation that will necessitate a prolonged recovery, compared to higher**  
168 **frequency fatigue.**<sup>17</sup> **Secondly, as motor unit discharge rarely exceeds 30Hz during**  
169 **voluntary contraction,**<sup>17</sup> **low frequency stimulus can be considered a more functionally**  
170 **relevant intervention. Finally, as TMG is a passive and peripheral measurement it will**  
171 **minimise confounding variables such as the variability of central control factors.** Whilst  
172 remaining secured in the same position as for PMT the participants received the fatigue  
173 intervention, which consisted of a 5 minute electrical stimulation of the right GM, to evoke  
174 fatigue. The stimulation protocol involved a train of 15 electrical pulses (1 every 100ms)  
175 with a 1 second gap before the start of each subsequent train. The protocol lasted 5 minutes  
176 and participants were asked to endure the maximum current they could, to ensure fatigue  
177 (~110 mA). The control intervention consisted of the same positioning but receiving no  
178 stimulation for a period of 5 minutes to account for the effect of time. Also in the same  
179 position, with the ankle placed at 90°, isometric MVC of the plantar flexors was measured,  
180 before and after both intervention and control, to assess whether fatigue occurred. Each

participant performed three 5 second MVCs, with 60 seconds recovery between attempts. Participants were provided with consistent verbal motivation to ensure maximal effort throughout.

*Statistical Analysis*

All data are presented as mean  $\pm$  SD. **After testing for assumption of normality of the dependent variables and log-transforming where necessary (i.e. when not normally distributed),** a 3 (measurements: before warm-up, M1; after warm-up, M2; after intervention, M3)  $\times$  2 (condition: control and fatigue intervention) ANOVA with repeated measures on both factors was used to detect differences in PMT and TMG parameters as a result of the fatigue/ control protocol. Where a significant F value was found a Tukey post hoc test was used to identify where any significant difference occurred. Paired *t-test* was conducted to compare the pre- / post-fatigue MVC difference between the control and fatigue intervention. Effect size (ES) was also calculated using eta-squared ( $\eta^2$ ) and interpreted as small (0.01), moderate (0.06) or large (0.14).<sup>22</sup> The percentage differences between control and fatigue intervention were also calculated and interpreted based on the minimum detectable change as reported in a previous reliability study.<sup>13</sup> An alpha level of  $p < 0.05$  was considered statistically significant. Statistical analysis was performed using Statistica version 10 (Statsoft LTD, Bedford, UK).

**Results**

*TMG parameters*

Dm demonstrated a fatigue-associated alteration. A significant main effect for 'condition' ( $F=7.2$ ,  $p=0.002$ ,  $\eta^2 = 0.27$ ) was documented for Dm, along with a post-hoc difference at M3 demonstrating that the fatigue condition was significantly lower than control condition ( $3.3 \pm 1.2$  vs  $4.0 \pm 1.4$  mm,  $p=0.031$ ; figure 4), with a percentage difference of 17.7%. No significant difference was found for any of the factors or their interaction for Vc, which exhibited  $121.8 \pm 43.2$  vs  $124.7 \pm 45.5$  mm·s<sup>-1</sup> at M1,  $121.3 \pm 45.7$  vs  $124.9 \pm 44.7$  mm·s<sup>-1</sup> at M2,  $131.3 \pm 44.6$  vs  $139.8 \pm 50.6$  mm·s<sup>-1</sup> at M3.

#### *MVC and PMT*

Plantar flexor isometric MVC exhibited a significant interaction 'condition x measurement' ( $F=12.4$ ,  $p=0.001$ ,  $\eta^2 = 0.91$ ) with post-hoc analysis showing a significant decline following the fatigue intervention ( $-122.6 \pm 104$  N;  $p<0.001$ ) but not following control ( $-25.7 \pm 71.3$  N,  $p=0.115$ ). The PMT exhibited a significant interaction 'condition x measurement' ( $F=5.9$ ,  $p=0.005$ ,  $\eta^2 = 0.23$ ). The post-hoc analysis revealed at M3 that fatigue caused significantly more tension than control ( $139.8 \pm 54.3$  vs.  $111.3 \pm 44.6$  N,  $p=0.007$ ; figure 5), with a percentage difference of 20.4%.

#### **Discussion**

This study was designed to evaluate the validity of TMG, as a sub-maximal assessment method, to detect local muscular fatigue, against functional physiological measures. Fatigue of the GM was achieved, as evidenced by the significant decline in peak force (MVC), which was absent following the control condition. This alteration in functional capacity of the muscle was associated with a significant decline in TMG Dm, similar to previous studies

224 following dynamic fatigue.<sup>16,23</sup> In addition, plantar flexor PMT increased following the  
225 fatigue intervention suggesting that the GM skeletal muscle-tendon unit became stiffer.  
226 Despite these alterations, muscle twitch Vc appeared to remain unaffected by fatigue.

227 When considering the physiological effects of fatigue there are a number of important  
228 variables to examine. We have previously demonstrated that during fatigued voluntary  
229 contractions muscle fibre conduction velocity declines due to a reduction in extracellular  
230 pH.<sup>24</sup> It is likely that this occurs due to a pH driven alteration of the Na<sup>+</sup> and K<sup>+</sup> gradient  
231 across the sarcolemma<sup>25</sup> and impairs action potential propagation. Therefore, during TMG  
232 measurement the electrical stimulus applied to the surface of the fatigued muscle should  
233 result in a slowing down of the action potentials propagated to reduce Ca<sup>2+</sup> release and  
234 subsequent excitation-contraction (E-C) coupling. Low-frequency fatigue, as characterized  
235 by a disproportionate reduction in force at lower stimulation frequencies, has been  
236 associated with E-C uncoupling.<sup>26</sup> It has been suggested that E-C uncoupling is attributable  
237 to, amongst other factors, impaired Ca<sup>2+</sup> transport via Ryanodine receptor channels in the  
238 triadic compartment.<sup>27</sup> Furthermore, other contributing factors will be from increased Pi  
239 which can push the cross-bridge into a low force generating status<sup>28</sup> and may also cause  
240 actin and myosin to detach.<sup>29</sup> These altered characteristics of muscle function will inevitably  
241 impair its force generation capacity, as shown by the significant decline in MVC.

242 **It has been reported previously that a stiffer muscle, as we have evidenced here by the**  
243 **rise in PMT (figure 5), will be associated with a reduced TMG Dm measurement.<sup>8</sup> In**  
244 **contrast to the current findings, Garcia-Manso et al<sup>15</sup> showed an *increase* in Biceps**  
245 **Femoris TMG Dm associated with fatigue following an ironman triathlon. The precise**  
246 **reasons for this disparity are unclear; however Morin, Tomazin, Edouard, & Millet<sup>30</sup>**

showed a small decline in whole leg stiffness during a running task, following a 24-hour marathon. These authors postulated that central fatigue would have been apparent which would have been linked to altered peripheral feedback from muscle afferents triggered from cytokines. This, we suggest, may be why an increase in TMG Dm was observed following an ironman triathlon when a decline has been reported with other types of fatigue from far shorter contractile/ exercise durations. Other studies have also demonstrated alterations in Dm alongside muscle architectural changes. Firstly, Pisot et al,<sup>8</sup> showed that following 35 days of bed rest, TMG Dm increased alongside the reduction in muscle thickness which the authors suggested would have contributed to reduced muscle stiffness. Secondly, we previously demonstrated<sup>31</sup> that altering the length of the muscle will determine the magnitude of TMG parameters, such that longer muscle length, as achieved through altered joint angle, results in reduced Dm. Thirdly, although not relating the decline in TMG Dm to muscle stiffness changes, other studies<sup>16,23</sup> have also demonstrated a decline in TMG Dm following fatigue, suggesting that this is an important parameter when assessing the muscle status in this regard.

In the present study we observed decreases in TMG Dm without significant alterations in Vc. Given previously described reductions in action potential propagation and muscle fibre conduction velocity, associated with fatigue,<sup>24</sup> it may have been expected that TMG Vc would be observed to decline post-fatigue, in concurrence with Dm. It is plausible that the lack of significant alteration in Vc is due to the high degree of inter-individual variability associated with the measurement. **Indeed, changes between measurements (M1, M2, M3) ranged from about -25% to +25% between participants.** The comparably low amplitude of the electrical stimulation used to elicit the peak TMG response, may perhaps render these

data difficult to compare to existing conduction velocity findings. As such, it may be inappropriate to consider alterations in the speed/ time component of the TMG response, when assessing muscle fatigue, with the focus instead being placed on spatial alterations (Dm), which we have shown here to be indicative of increased muscle stiffness.

As with any type of physiological measurement there will be a degree of variability. We have previously accounted for this variability with TMG measured under different muscle conditions<sup>13</sup> and shown Dm to be well within acceptable limits. Analogous to this is establishing minimal detectable change so practitioners and researchers can be confident that the given magnitude of observed change following any intervention is real and physiologically significant. We have demonstrated in this study that the fatigue-altered Dm parameter (17.7%) clearly exceeds the minimal detectable change thresholds of 15.1%.<sup>13</sup> Furthermore, the effect size for the data presented in this study, as described by Cohen,<sup>22</sup> is “large” suggesting that this particular TMG measure is sufficiently sensitive to adequately detect local muscular fatigue. **Nonetheless, a number of limitations must be considered. Current findings can only be applied to a healthy, young male population. It remains to be seen whether TMG measurements are sufficiently sensitive to detect fatigue associated changes in alternative cohorts. Additionally, GM was selected for investigation as its anatomical position facilitates measurement using TMG. Muscles which are not located superficially, but may still be of interest, are not measureable using the methods described herein.**

## **Conclusion**

This is the first study to demonstrate that TMG was effective in detecting local muscular fatigue in the GM. We propose that this response was directly related to increased stiffness

of the muscle from impaired contractile capacity. It should be emphasised that, when assessing local muscular fatigue, Dm of the muscle is a valid measure, **however it remains to be seen whether TMG has the sensitivity to detect any changes in Vc in a different context.** The current findings have important implications for researchers and practitioners seeking to establish fatigue status of skeletal muscle, with implications for prevention of **over-training injuries** in sports-related activities. Given the **non-invasive and sub-maximal** nature of this type of measurement, TMG can be used to determine local muscular fatigue in patients who may be unable to exert the maximal effort required for voluntary muscle function assessments. **Additionally, TMG measurements are exempt from the bias of volitional effort and motivation, facilitating the incorporation of the procedure into existing programmes.**<sup>32</sup> Furthermore, TMG could be utilised regularly, as a monitoring tool, without fear of detriment to muscle function.

#### Acknowledgements

[REDACTED]

#### References

1. Garrandes, F., Colson, S. S., Pensini, M., Seynnes, O., & Legros, P. Neuromuscular fatigue profile in endurance-trained and power-trained athletes. *Medicine and Science in Sports and Exercise*, 2007; 39 (1): 149–158.
2. Ditroilo, M., Watsford, M., Fernandez-Pena, E., D'Amen, G., Lucertini, F., & De Vito, G. Effects of fatigue on muscle stiffness and intermittent sprinting during cycling. *Medicine & Science in Sports & Exercise*, 2011; 43 (5): 837–845.

- 315 3. Babault, N., Desbrosses, K., Fabre, M.-S., Michaut, A., & Pousson, M. Neuromuscular  
316 fatigue development during maximal concentric and isometric knee extensions. *Journal of*  
317 *Applied Physiology*, 2006; 100 (3): 780–785.
- 318 4. Berchicci, M., Menotti, F., Macaluso, A. & Di Russo, F. The neurophysiology of central and  
319 peripheral fatigue during sub-maximal lower limb isometric contractions. *Frontiers in*  
320 *Human Neuroscience*, 2013; 7 (135).
- 321 5. Graven-Nielsen, T., Lund, H., Arendt-Nielsen, L., Danneskiold-Samsøe, B., & Bliddal, H.  
322 Inhibition of maximal voluntary contraction force by experimental muscle pain: a centrally  
323 mediated mechanism. *Muscle & nerve*, 2002; 26(5): 708-712.
- 324 6. Dahmane, R., Valenčič, V., Knez, N., & Erzen, I. Evaluation of the ability to make  
325 noninvasive estimation of muscle contractile properties on the basis of the muscle belly  
326 response. *Medical & Biological Engineering & Computing*, 2001; 39: 51–55.
- 327 7. Dahmane, R., Djordjevic, S., Simunič, B., Valenčič, V. Spatial fiber type distribution in  
328 normal human muscle Histochemical and tensiomyographical evaluation. *Journal of*  
329 *Biomechanics*, 2005; 38: 2451–2459.
- 330 8. Pisot, R., Narici, M. V, Simunič, B., De Boer, M., Seynnes, O., Jurdana, M., Biolo, G., &  
331 Mekjavić, I. B. Whole muscle contractile parameters and thickness loss during 35-day bed  
332 rest. *European Journal of Applied Physiology*, 2008; 104 (2): 409–414.
- 333 9. Orizio, C. Surface Mechanomyogram. In M. Roberto & P. P. A (Eds.), *Electromyography:*  
334 *Physiology, Engineering and Noninvasive Applications* (pp. 305–322). Hoboken: John Wiley &  
335 Sons; 2004.



- 336 10. Tous-Fajardo, J., Moras, G., Rodriguez-Jimenez, S., Usach, R., Doutres, D. M., &  
337 Maffiuletti, N. A. Inter-rater reliability of muscle contractile property measurements using  
338 non-invasive tensiomyography. *Journal of Electromyography and Kinesiology*, 2010; 20 (4):  
339 761–766.
- 340 11. Krizaj, D., Simunič, B., & Zagar, T. Short-term repeatability of parameters extracted from  
341 radial displacement of muscle belly. *Journal of Electromyography and Kinesiology*, 2008; 18  
342 (4): 645–651.
- 343 12. Simunič, B. Between-day reliability of a method for non-invasive estimation of muscle  
344 composition. *Journal of Electromyography and Kinesiology*, 2012; 22 (4): 527–530.
- 345 13. Ditroilo, M., Smith, I. J., Fairweather, M. M., & Hunter, A. M. Long-term stability of  
346 tensiomyography measured under different muscle conditions. *Journal of Electromyography  
347 and Kinesiology*, 2013; 23 (3): 558–563.
- 348 14. Hunter, A. M., Galloway, S. D. R., Smith, I. J., Tallent, J., Ditroilo, M., Fairweather, M. M.,  
349 & Howatson, G. Assessment of eccentric exercise-induced muscle damage of the elbow  
350 flexors by tensiomyography. *Journal of Electromyography and Kinesiology*, 2012; 22 (3):  
351 334–341.
- 352 15. Garcia-Manso, J. M., Rodriguez-Ruiz, D., Rodriguez-Matoso, D., de Saa, Y., Sarmiento, S.,  
353 & Quiroga, M. Assessment of muscle fatigue after an ultra-endurance triathlon using  
354 tensiomyography (TMG). *Journal of Sports Sciences*, 2011; 29 (6): 619–625.
- 355 16. Garcia-Manso, J. M., Rodriguez-Matoso, D., Sarmiento, S., de Saa, Y., Vaamonde, D.,  
356 Rodriguez-Ruiz, D., & Da Silva-Grigoletto, M. E. Effect of high-load and high-volume

- 357 resistance exercise on the tensiomyographic twitch response of biceps brachii. *Journal of*  
358 *Electromyography and Kinesiology*, 2012; 22 (4): 612–619.
- 359 17. Allen, D. G., Lamb, G. D., & Westerblad, H. Skeletal muscle fatigue: cellular mechanisms.  
360 *Physiological Reviews*, 2008; 88 (1): 287–332.
- 361 18. Paasuke, M., Saapar, L., Ereline, J., Gapeyeva, H., Requena, B., & Oopik, V. Postactivation  
362 potentiation of knee extensor muscles in power- and endurance-trained, and untrained  
363 women. *European Journal of Applied Physiology*, 2007; 101 (5): 577–585.
- 364 19. Valenčič, V., & Knez, N. Measuring of skeletal muscles' dynamic properties. *Artificial*  
365 *Organs*, 1997; 21 (3): 240-242.
- 366 20. Fowles, J. R., Sale, D. G., & MacDougall, J. D. Reduced strength after passive stretch of  
367 the human plantarflexors. *Journal of Applied Physiology*, 2000; 89 (3): 1179–1188.
- 368 **21. Araújo, V. L., Carvalhais, V. O. C., Souza, T. R., Ocarino, J. M., Gonçalves, G. G. P., &**  
369 **Fonseca, S. T. Validity and reliability of clinical tests for assessing passive ankle stiffness.**  
370 ***Revista Brasileira de Fisioterapia*, 2011; 15 (2): 166-173.**
- 371 22. Cohen, J. *Statistical Power Analysis for the Behavioural Sciences* (2nd ed.). Hillsdale, NJ:  
372 Lawrence Erlbaum Associates; 1988.
- 373 23. Carrasco, L., Sanudo, B., de Hoyo, M., Pradas, F., & Da Silva, M. E. Effectiveness of low-  
374 frequency vibration recovery method on blood lactate removal, muscle contractile  
375 properties and on time to exhaustion during cycling at VO<sub>2</sub>max power output. *European*  
376 *Journal of Applied Physiology*, 2011; 111 (9): 2271–2279.

- 377 24. Hunter, A. M., De Vito, G., Bolger, C., Mullany, H., & Galloway, S. D. R. The effect of  
378 induced alkalosis and submaximal cycling on neuromuscular response during sustained  
379 isometric contraction. *Journal of Sports Sciences*, 2009; 27 (12): 1261–1269.
- 380 25. Brody, L. R., Pollock, M. T., Roy, S. H., De Luca, C. J., & Celli, B. pH-induced effects on  
381 median frequency and conduction velocity of the myoelectric signal. *Journal of Applied*  
382 *Physiology*, 1991; 71 (5): 1878–1885.
- 383 26. Hill, C. A., Thompson, M. W., Ruell, P. A., Thom, J. M. & White, M. J. Sarcoplasmic  
384 reticulum function and muscle contractile character following fatiguing exercise in humans.  
385 *Journal of Physiology*, 2001; 531: 871-878.
- 386 27. Balog, E. M. Excitation-contraction coupling and minor triadic proteins in low-frequency  
387 fatigue. *Exercise and Sport Sciences Reviews*, 2010; 38 (3): 135-142.
- 388 28. Nocella, M., Colombini, B., Benelli, G., Cecchi, G., Bagni, M. A., & Bruton, J. Force decline  
389 during fatigue is due to both a decrease in the force per individual cross-bridge and the  
390 number of cross-bridges. *The Journal of Physiology*, 2011; 589 (Pt 13): 3371–3381.
- 391 29. Takagi, Y., Shuman, H., & Goldman, Y. E. Coupling between phosphate release and force  
392 generation in muscle actomyosin. *Philosophical Transactions of the Royal Society of London.*  
393 *Series B, Biological Sciences*, 2004; 359 (1452): 1913–1920.
- 394 30. Morin, J. B., Tomazin, K., Edouard, P., & Millet, G. Y. Changes in running mechanics and  
395 spring-mass behaviour induced by a mountain ultra-marathon race. *Journal of*  
396 *Biomechanics*, 2011; 44 (6): 1104–1107.

397 31. Ditroilo, M., Hunter, A. M., Haslam, S., & De Vito, G. The effectiveness of two novel  
398 techniques in establishing the mechanical and contractile responses of biceps femoris.  
399 *Physiological Measurement*, 2011; 32 (8): 1315–1326.

400 32. Rey, E., Lago-Peñas, C., & Lago-Ballesteros, J. Tensiomyography of selected lower-limb  
401 muscles in professional soccer players. *Journal of Electromyography and Kinesiology*, 2012;  
402 22 (6): 866-872.

For Peer Review

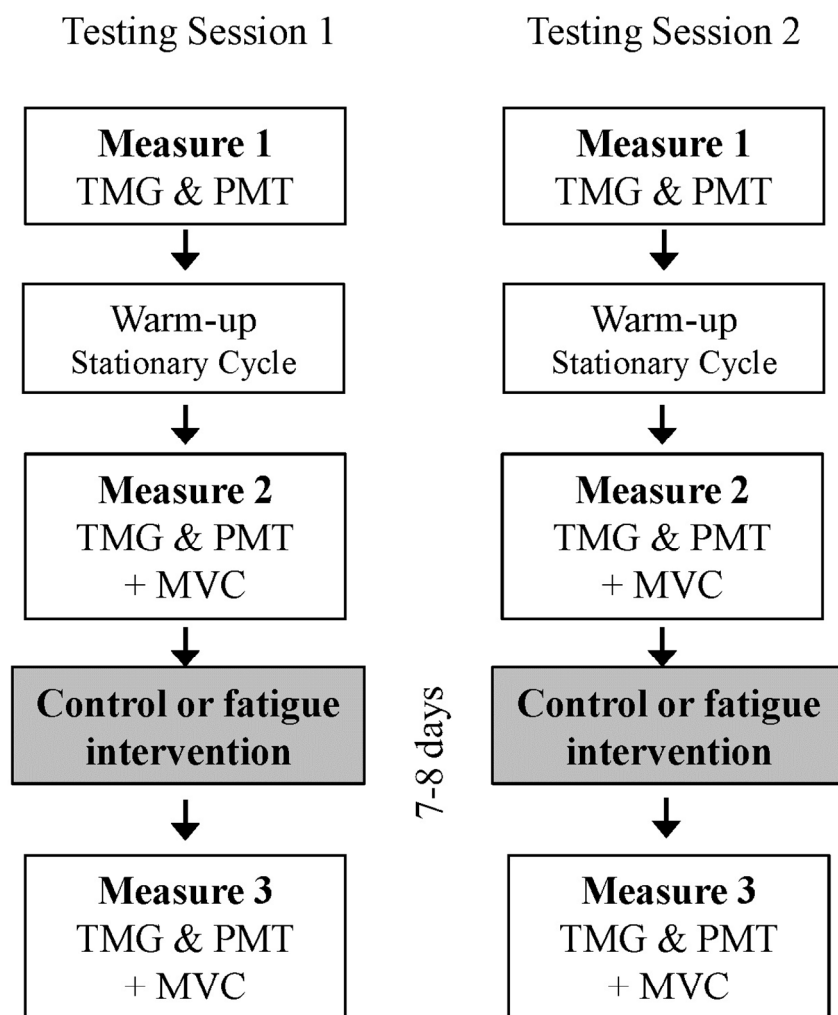


Figure 1. Schematic representation of the research design. TMG = Tensiomyography; PMT = passive muscle tension.

120x150mm (300 x 300 DPI)

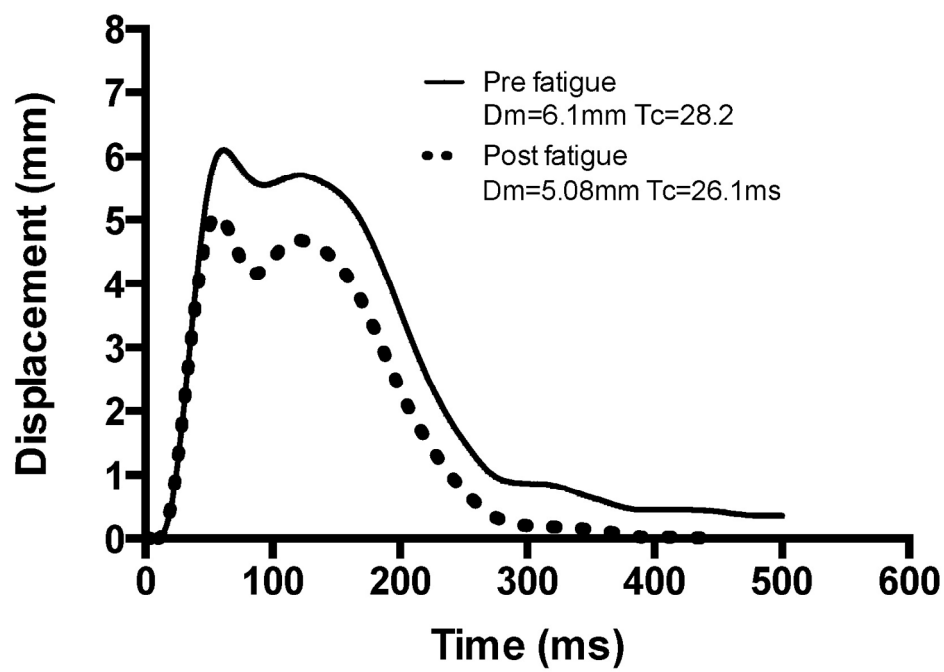


Figure 2. Typical displacement/ time curve of the tensiomyographic signal before and after the administration of the fatigue protocol. Dm = muscle displacement; Tc = contraction time.  
150x110mm (300 x 300 DPI)



Figure 3. Isokinetic dynamometer setup for PMT and MVC assessment. Ankle flexed at 90° relative to the tibia.  
342x192mm (300 x 300 DPI)

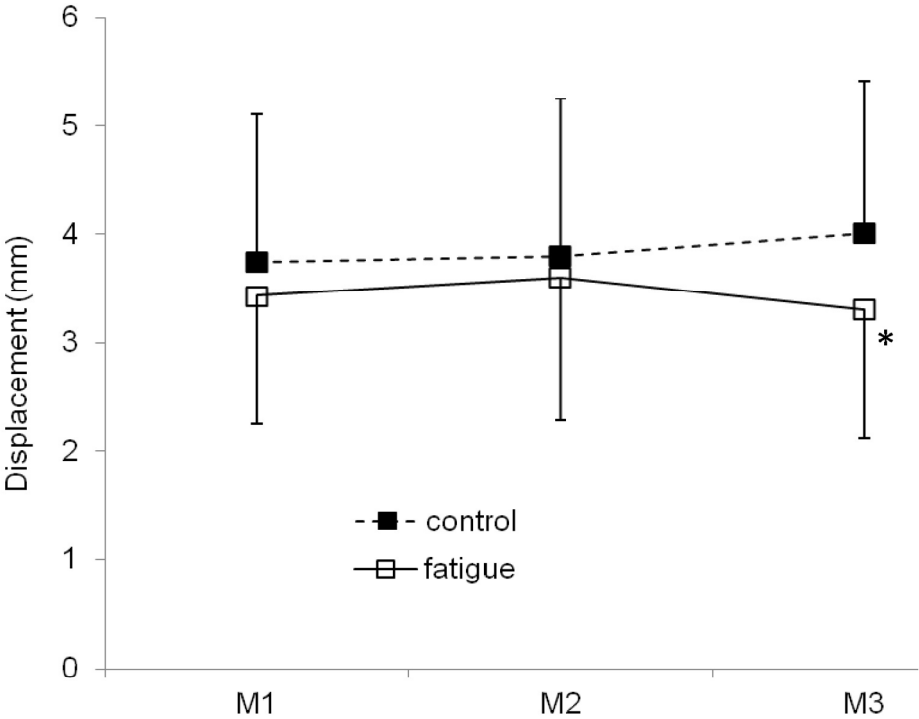


Figure 4. Average values (+ SD) of passive muscle tension as assessed on the isokinetic dynamometer at the three measurement points. \* = significant different from 'control' at M3,  $p < 0.01$ .  
442x383mm (96 x 96 DPI)



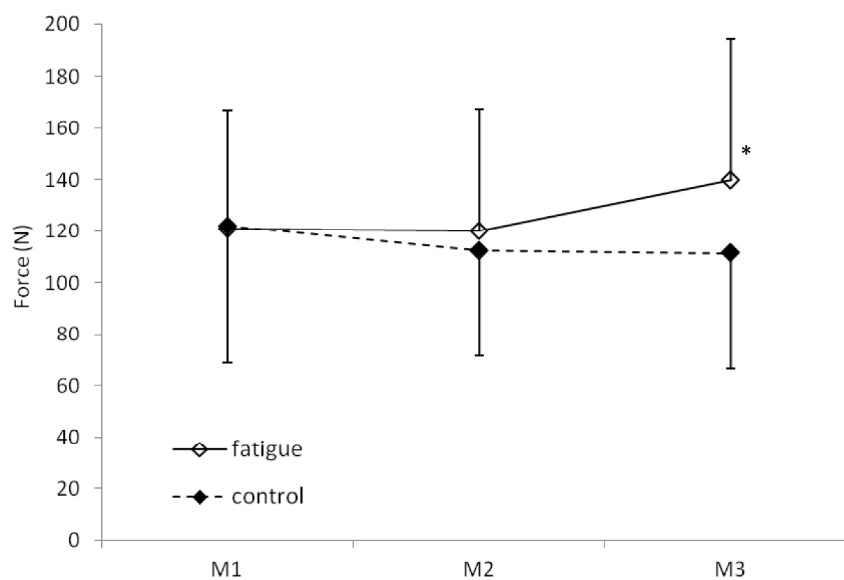


Figure 5. Average values (+ SD) of muscle displacement as assessed by tensiomyography at the three measurement points. \* = significant different from 'control' at M3,  $p < 0.05$ .  
254x190mm (300 x 300 DPI)